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Association between polymorphisms of *CYP19*, *CYP21*, and *ER1* genes and milk production traits in Black-and-White cattle

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Abstract: The relationships between the SNPs of the cytochrome P450 gene (*CYP19*/*Pvu*II), steroid 21-hydroxylase (P450c21) gene (*CYP21/Hpa*II), 2 polymorphic sites of estrogen receptor alpha gene (*ER1/Bgl*I and *ER1/Sna*BI), and milk production traits of Black-and-White cattle were analyzed. A total of 472 cows were included in the study and genotyped using PCR-RFLP. The frequencies of alleles for the Black-and-White cows were as follows: 0.923 - $CYP19^{A}$, 0.077 - $CYP19^{B}$, 0.042 - $ER1/BglI^{A}$, and 0.958 for $ER1/BglI^{G}$. For *ER1/Sna*BI, the frequency of allele *A* was 0.960 and that of allele *G* was 0.040. In the *CYP21*, all cows were genotyped as *AA* (no polymorphism). There were no associations between *CYP19*/*Pvu*II, *CYP21/Hpa*II, *ER1/Bgl*I, *ER1/Sna*BI polymorphisms, and milk production traits of the investigated cows.

Key words: *ER1* gene, *CYP19* gene, *CYP21* gene, milk production traits, dairy cattle

Introduction

The genes of cytochromes P450 are the part of the multigene superfamily, which contains 27 distinct gene families. Ten of those are specific for mammals (1). Two of them are *CYP19* and *CYP21* gene families.

Aromatase cytochrome P450 enzyme is encoded by the *CYP19* gene (2,3). The role of aromatase, which is part of the aromatase enzyme complex, is the conversion of androgens to estrogens, and is essential for physiology of reproduction (4). Bovine *CYP19* was mapped to band q2.6 on chromosome 10 (5). *CYP19* utilizes different promoters in tissue-specific expression in the mechanism of alternative splicing (6). Different promoter regions correspond to different 5'-UTR transcripts but the coding region is identical for all tissues (7). In placenta, expression of aromatase is mainly driven by P1.1, a distal promoter (8). The role of *CYP19* for milk production traits arises from the fact that estrogen is involved (not directly) in lactogenesis. It influences mammary cells by increasing the numbers of prolactin and growth hormone receptors.

In this study, we analyzed polymorphisms in the promoter 1.1 region of the bovine *CYP19* gene (GenBank no. Z69241). The polymorphic site was located at 1044 nt in P1.1 and the A→G transition was recognized by *Pvu*II restriction endonuclease (3,9).

CYP21 gene encodes the steroid 21-hydroxylase (P450c21), the enzyme necessary in corticosteroids metabolism (10) and involved in steroidogenesis in the

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adrenal cortex (11). Enzyme (P450c21) is connected with microsomal P450 cytochrome (10). *CYP21* is a candidate gene due to several quantitative trait loci (QTL) for reproductive traits such as ovulation rate and, as we suspect, due to milk production traits, as well.

The gene coding 21-hydroxylase is one of the most polymorphic genes in humans. There are over 50 described mutations within *CYP21*. Most of those mutations are caused by micro- or macro-conversions of *CYP21* with non-coding inactive pseudogene *CYP21P* (10).

Bovine *CYP21* gene, mapped on chromosome 23, contains 10 exons. Cattle have 2 copies of this gene and synthesize 2 sizes of P450c21 mRNA in the adrenal cortex (12). A high polymorphism, in different cattle breeds, was shown for *CYP21*. Within the promoter region of the bovine *CYP21* gene, the Bov-A2 SINEs (short interspersed nucleotide element) are located (13). The Bov-A2 is a homodimer of 115 bp segments and is specific for *Bovidae* genomes (14). Those kinds of retroelements might be one of the most important sources of single nucleotide polymorphism (14). In this study, we analyzed the region of the bovine *CYP21* gene (GenBank no. M11267, AF163098, AF163767) and we predicted to find polymorphic site within the promoter region of *CYP21* gene recognized by *Hpa*II restriction endonuclease.

Estrogens play a crucial role in physiology of reproduction, cell growth, differentiation, mammary gland development, milk synthesis and also in oncogenesis. Due to the numerous functions that estrogens play in organisms of farm animals, their genes are considered markers for production traits (15). Other genes, which can be used as markers for milk production, are the estrogen receptor α (*ER1*) and β (*ER2*) genes (16). Estrogen receptors are mediators in estrogen actions by the transcription of target genes. It is known that ERs are transcription factors, which, after binding a proper ligand, e.g. 17β-estradiol, are capable of regulation of genes transcription (15,17).

The bovine *ER1* gene is located on chromosome 6 and the protein is coded by 8 exons. The 5' region of *ER1* has additional exons that do not code for protein but code for transcripts of different lengths with different 5'UTRs. The alternative exons are spliced to the +85 acceptor site located in the coding exon 1. The functions of the different *ER1* transcripts are unknown but they are present in specific tissues and at specific stages of development (17).

In the present study, we analyzed polymorphisms in the 5' region of the bovine *ER1* gene (GenBank no. AY340597), an A/G transition upstream to exon C, identified for the first time by Szreder and Zwierzchowski (15). To recognize the A/G transition, *Bgl*I restriction endonuclease was used (15).

The second polymorphic site (A→G transition) for *ER1* (GenBank no. AY332655) analyzed in the present study was a part of the putative promoter for exon B within the 5' region of the bovine *ER1* gene at position -1213 relative to the +85 splicing acceptor site in exon 1 (17). The *Sna*BI restriction nuclease was used for genotyping.

We consider that the polymorphisms in specific placental promoter P1.1 of *CYP19*, polymorphism in specific 5' region of *CYP21*, and polymorphic 5' regions of *ER1* in cattle could influence milk production traits.

The aim of this study was to identify the polymorphism of the 5' promoter regions of the 3 genes (*CYP19*, *CYP21*, and *ER1*) in Black-and-White (B&W) cattle and their associations with milk production traits.

Materials and methods

A total of 472 B&W cows were analyzed. The individuals were kept in West Pomeranian region of Poland. The 4 genotypes: *CYP19/Pvu*II, *CYP21/Hpa*II, *ER1/Bgl*I, and *ER1/Sna*BI were analyzed using PCR-RFLP (3,15,17). The genomic DNA was isolated from blood samples using a *Master Pure*^{TM} Kit (Epicentre Technologies). The analyzed fragments of the 3 genes were amplified using the primers presented in Table 1. The primer sequences for *CYP21* were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/). The PCR reactions contained approx. 90-100 ng of genomic DNA, 0.5 μM of each primer, 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP, 0.5 units of *Taq* polymerase (MBI Fermentas) and deionized water up to 15 μL. The following numbers of cycles for PCR reactions were applied: 35, 30, 28, and 35 for *CYP19*, *CYP21*, *ER1-Bgl*I, and *ER1-Sna*BI, respectively.

Table 1. Primers and PCR conditions used for genotyping of the bovine CYP19, CYP21 and ER1 genes.

Gene/ Polymorphic site	Primers sequences (5'-3')	Tm (°C)	Length (bp)	References
*CYP19/Pvu*II	forward 5'-CTCTCGATGAGACAGGCTCC-3' reverse 5'-ACAATGCTGGGTTCTGGACT-3'	59	405	(3)
*CYP21/Hpa*II	forward 5'-TGTAAGATGAGTGCCGGAGA-3' reverse 5'-TCTGTGCGACCCCATAGAT-3'	60	252	-
*ER1/Bgl*I	forward 5'-TTTGGTTAACGAGGTGGAG-3' reverse 5'-TGTGACACAGGTGGTTTTC-3'	53	242	(15)
*ER1/Sna*BI	forward 5'-GTCAGGTATTCCGTCAGGT-3' reverse 5'-GCCTTTCTGTTCCTTTGG-3'	54	340	(17)

The PCR products of 3 genes were digested with enzymes (Fermentas UAB, Vilnius, Lithuania) at 37 °C/3 h (Table 2). The digestion products were separated by horizontal electrophoresis (120 V, 50 min for *CYP19* and *CYP21* and 90 V, 70 min for *ER1*) in 2% agarose gels (PRONA) in 1 × TBE and 1.0 μM ethidium bromide (AppliChem, LLC).

The data of milk production traits in the 1st, 2nd, and 3rd lactations were obtained from the farm documentation. The statistical model used was as follows:

$$Y_{ijkl} = \mu + G_i + s_j + YS_k + \alpha(h_i - h_m) + \beta(w_i - w_m) + e_{ijkl},$$

where: Y_{ijkl} – examined trait, μ – overall mean, G_i – effect of genotype, s_j – random effect of a sire, YS_k – effect of calving year-season, α – regression coefficient for the proportion of HF genes in a cow genotype, h_i – proportion of HF genes in the genotype of cow i, h_m – average proportion of HF genes in the population, β – regression coefficient for the cow age, w_i – age of cow *i*, w_m – average cow age in the population, e_{ijkl} – random error.

Results

In the group of B&W cows, the following DNA restriction fragments were obtained for the *CYP19/Pvu*II polymorphism: 405 bp for the *AA* genotype (no digestion); 405, 327, and 78 bp for *AB*; and 327 and 78 bp for *BB* genotype (Figure). All individuals were genotyped as *AA* (no digestion) for the *CYP21/Hpa*II polymorphism (Table 3). For the *ER1/Bgl*I polymorphism, 3 genotypes were obtained: *GG* (182 and 60 bp), *AG* (242, 182 and 60 bp), and *AA* (242 bp, no digestion), and for *ER1/Sna*BI polymorphism only 2 genotypes were present: *AA* (340 bp, no digestion) and *AG* (340, 225, 115 bp).

Table 2. Restriction endonucleases used for genotyping and digestion of the products of *CYP19*, *CYP21*, and *ER1* genes.

Genes	Enzymes	Genotypes/Digestion products		Sites/sequences of digestion
CYP19	*Pvu*II	*AA* *AB* *BB*	405 bp (no digestion) 405, 327, 78 bp 327, 78 bp	5'-C A G^C T G-3' 3'-G T C^G A C-5'
CYP21	*Hpa*II	*AA*	252 bp (no digestion)	5'-C^C G G-3' 3'-G G C^C-5'
ER1	*Bgl*I	*AA* *GA* *GG*	242 bp (no digestion) 242, 182, 60 bp 182, 60 bp	5'-G C C N N N N^N G G C-3' 3'-C G G N^N N N N C C G-5'
ER1	*Sna*BI	*AA* *GA* *GG*	340 bp (no digestion) 340, 225, 115 bp 225, 115 bp	5'-T A C^G T A-3' 3'-A T G^C A T-5'

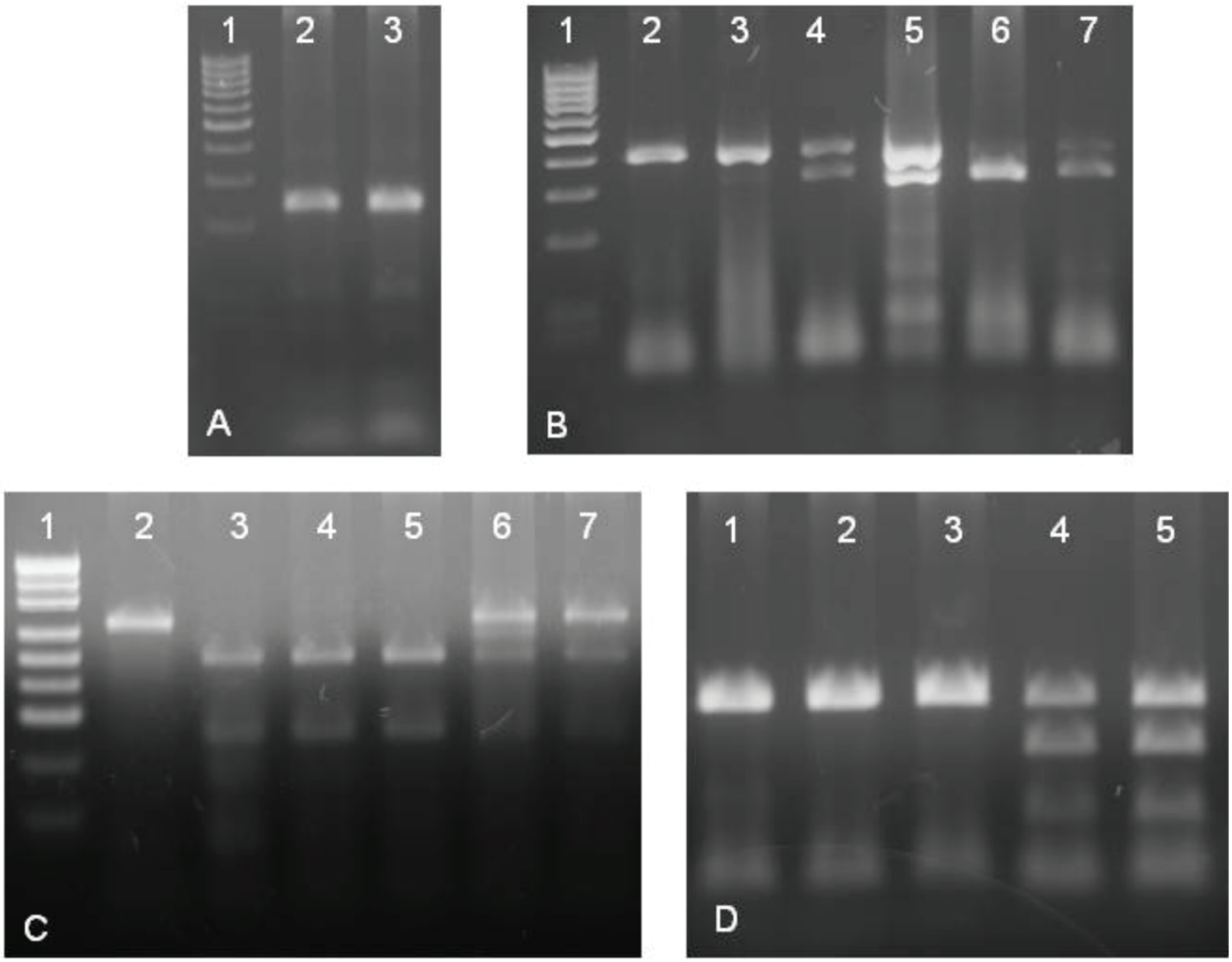

Figure. The results of the PCR –RFLP analysis:
A) *CYP21*/*Hpa*II: 1 – mass marker GeneRuler™ 50 bp DNA Ladder; 2 – PCR product (252 bp); 3 – *AA* genotype (252 bp); **B)** *CYP19*/*Pvu*II: 1 – mass marker GeneRuler™ 50 bp DNA Ladder; 2, 3 – *AA* genotype (405 bp); 4, 5 – *AB* genotype (restriction fragments 405, 327, and 87 bp, respectively); 6, 7 – *BB* genotype (restriction fragments 327 and 78 bp, respectively); **C)** *ER1*/*Bgl*I: 1 – mass marker pUC19 DNA/*Msp*I (*Hpa*II) Marker, 23; 2 – PCR product and *AA* genotype (242 bp); 3, 4, 5 – *GG* genotype (restriction fragments 182 and 60 bp, respectively); 6, 7 – *AG* genotype (restriction fragments 242, 182, and 60 bp, respectively); **D)** *ER1*/*Sna*BI: 1 – PCR product (340 bp); 2, 3 – *AA* genotype (restriction fragment 340 bp); 4, 5 – AG genotype (restriction fragments 340, 225, and 115 bp, respectively).

Table 3 shows the frequencies of genotypes and alleles of the 4 gene polymorphisms obtained in this study.

Tables 4 and 5 show the relationships between the polymorphisms of the *CYP19* and *ER1* genes and milk production traits in B&W cattle. The analysis indicated that the level of the analyzed traits was significantly influenced by the year/season, sire, proportion of Holstein-Friesian (HF) genes, and age. The *CYP21*/*Hpa*II was excluded from the statistical analysis due to its monomorphism.

The influence of the *CYP19*/*Pvu*II genotype on milk yield was greatest in the 3rd lactation and smallest in the 1st lactation but no statistically significant differences were found. Similarly, *CYP19*/*Pvu*II influence on the protein yield was greatest in the 3rd and smallest in the 2nd lactation. The genotype influence on fat yield, fat content, and protein content was greatest in the 2nd lactation but no statistically significant differences were noticed. The analysis showed that the milk and protein yields in the 3rd lactation were significantly influenced

Table 3. Frequencies of genotypes and alleles of *CYP19/Pvu*II, *CYP21/Hpa*II, *ER1/Bgl*I and *ER1/Sna*BI.

Polymorphism	Genotypes			Alleles	
*CYP19/Pvu*II	*AA*	*AB*	*BB*	$CYP19^A$	$CYP19^B$
	0.850 (n = 401)	0.146 (n = 69)	0.004 (n = 2)	0.923	0.077
*CYP21/Hpa*II	*AA*	*AB*	*BB*	$CYP21^A$	$CYP21^B$
	(n = 472)	-	-	1.000	-
*ER1/Bgl*I	*AA*	*AG*	*GG*	ER^A	ER^G
	0.002 (n = 1)	0.080 (n = 38)	0.918 (n = 433)	0.042	0.958
*ER1/Sna*BI	*AA*	*AG*	*GG*	ER^A	ER^G
	0.920 (n = 434)	0.080 (n = 38)	-	0.960	0.040

Table 4. Significance of the influence of factors covered by statistical model on the examined traits (F values).

Character DF (1st/2nd/3rd lactation)	*CYP19/Pvu*II				
	Genotype	Year season	Sire	HF genes	Age
Milk yield (kg):					
1st lactation	1.15	1.12	1.42*	4.38**	2.74
2nd lactation	0.19	1.12	1.30	2.68	4.06*
3rd lactation	1.97	2.26**	1.52	2.98	1.56
Fat yield (kg):					
1st lactation	0.67	0.94	2.01**	11.31**	1.21
2nd lactation	2.29	0.99	1.46*	3.33	3.62
3rd lactation	1.70	1.52	1.12	2.93	0.70
Fat content (%):					
1st lactation	0.42	1.34	1.36*	2.19	0.90
2nd lactation	2.33	1.01	1.06	0.01	0.02
3rd lactation	0.02	1.09	0.90	0.02	0.09
Protein yield (kg):					
1st lactation	0.88	1.17	1.76**	14.50**	0.93
2nd lactation	0.04	1.12	1.50*	1.90	4.01
3rd lactation	2.23	2.55**	1.53	3.66	6.02*
Protein content (%):					
1st lactation	1.06	1.08	1.20	0.32	0.23
2nd lactation	1.32	1.50*	1.24	0.81	0.48
3rd lactation	0.12	1.02	1.13	0.00	5.20*

* - significance of differences at $P \leq 0.05$; ** - significance of differences at $P \leq 0.01$.

Table 5. Significance of the influence of factors covered by statistical model on the examined traits (F values).

Character DF (1st/2nd/3rd lactation)	*ER1/Bgl*I					*ER1/Sna*BI				
	Genotype	Year season	Sire	HF genes	Age	Genotype	Year season	Sire	HF genes	Age
Milk yield (kg):										
1st lactation	1.18	0.97	2.05**	10.35**	1.40	1.68	0.98	2.05**	10.76**	1.52
2nd lactation	0.32	1.13	1.35	3.22	4.20*	2.73	1.16	1.38*	3.14	4.85*
3rd lactation	0.57	2.15	1.55	3.21	1.45	0.09	2.13*	1.50	3.54	1.43
Fat yield (kg):										
1st lactation	0.60	1.10	1.43**	3.60	3.20	0.43	1.11	1.43**	3.72	3.34
2nd lactation	1.36	0.97	1.45*	4.82*	4.13*	3.46	0.99	1.47*	4.33*	4.61*
3rd lactation	0.49	1.52	1.12	3.15	0.64	0.26	1.52	1.09	3.41	0.67
Fat content (%):										
1st lactation	0.23	1.35	1.37*	2.50	1.11	0.59	1.36	1.37*	2.64	1.10
2nd lactation	0.67	0.94	1.02	0.05	0.00	0.04	0.93	1.01	0.01	0.00
3rd lactation	0.02	1.12	0.02	0.02	0.09	0.01	1.12	0.92	0.01	0.08
Protein yield (kg):										
1st lactation	1.42	1.19	1.81**	13.57**	1.14	0.87	1.19	1.80**	13.94**	1.23
2nd lactation	0.20	1.14	1.53*	2.14	4.17*	1.57	1.15	1.55**	2.08	4.63*
3rd lactation	0.21	2.44**	1.57*	4.07*	5.60*	0.18	2.44**	1.55	4.28*	5.66*
Protein content (%):										
1st lactation	0.04	1.08	1.18	0.34	0.18	1.06	1.10	1.19	0.31	0.19
2nd lactation	0.62	1.48	1.26	1.42	0.46	1.72	1.51*	1.26	1.21	0.57
3rd lactation	0.32	1.05	1.13	0.00	5.20*	0.25	1.03	1.12	0.01	5.46*

* - significance of differences at $P \leq 0.05$; ** - significance of differences at $P \leq 0.01$.

by the year/season. The proportion of HF genes influenced most significantly milk, fat, and protein yields in the 1st lactation.

The influence of the *ER1/Bgl*I and *ER1/Sna*BI genotypes on the analyzed traits was weak. The significant influence of the year/season on the protein yield in the 3rd lactation was found for the 2 *ER1* genotypes. The sire influenced significantly milk yield in the 1st lactation, fat yield in the 1st and 2nd lactations and protein yield in the 1st and 2nd lactation. Inclusion of the HF genes proportion in the models was statistically significant for the milk and protein yield in the 1st lactation and fat and protein yield in the 2nd and 3rd lactation. The effect of age was significant for the milk yield in the 2nd lactation as well as for the fat yield, protein yield, and content in the last 2 lactations.

Tables 6 and 7 show the influence of *CYP19*, *ER1/Bgl*I, and *ER1/Sna*BI genes polymorphisms on production traits in B&W cattle.

As it can be seen from Table 6, the milk yields of individuals of different *CYP19/Pvu*II genotypes were highest in the 1st and 3rd lactation for *AB* genotypes and in the 2nd lactation for *AA* genotype; however, no statistically significant differences were noticed. Due to the low number of individuals genotyped as homozygote *BB*, the lowest values of milk yield were observed in all 3 lactations. The highest levels of fat and protein yields were present for *AA CYP19/Pvu*II genotypes in all lactations but those differences were not statistically significant. The differences between fat/protein yields of *AA* and *AB CYP19/Pvu*II genotypes were small.

Table 6. Means and standard deviations of milk production traits in cows of different *CYP19/Pvu*II genotypes.

Lactation	*CYP19/Pvu*II						
	Genotype	n	Milk yield (kg)	Fat		Protein	
				kg	%	kg	%
I	*AA*	401	8468 (1353.5)	362.4 (56.2)	4.305 (0.446)	281.5 (41.7)	3.336 (0.196)
	AB	69	8495 (1611.0)	356.0 (74.8)	4.212 (0.527)	280.3 (50.7)	3.319 (0.218)
	BB	2	6048 (335.8)	258.5 (9.2)	4.270 (0.084)	201.0 (21.2)	3.315 (0.162)
II	*AA*	241	10810 (1922.4)	455.0 (82.7)	4.234 (0.552)	352.3 (60.7)	3.261 (0.184)
	AB	43	10587 (2145.3)	434.6 (84.8)	4.164 (0.636)	349.3 (65.8)	3.317 (0.200)
	BB	1	8116 (0.0)	374.0 (0.0)	4.600 (0.0)	269.0 (0.0)	3.310 (0.0)
III	*AA*	121	11235 (2342.4)	468.4 (103.0)	4.188 (0.552)	366.1 (76.6)	3.263 (0.215)
	AB	20	11460 (2486.6)	464.9 (91.9)	4.117 (0.664)	362.5 (63.1)	3.194 (0.219)
	BB	2	9822 (226.9)	451.0 (18.4)	4.605 (0.162)	310.5 (21.9)	3.165 (0.247)

Table 7. Means and standard deviations of milk production traits in cows of different *ER1/Bgl*I and *ERI/Sna*BI genotypes.

L	*ER1/Bgl*I							*ER1/Sna*BI						
	Genotype	n	Milk yield (kg)	Fat		Protein		Genotype	n	Milk yield (kg)	Fat		Protein	
				kg	%	kg	%				kg	%	kg	%
I	*GG*	433	8467 (1403.1)	361.4 (59.7)	4.293 (0.460)	281.3 (43.5)	3.334 (0.201)	-	-	-	-	-	-	-
	AG	38	8414 (1380.3)	357.2 (58.1)	4.269 (0.445)	278.9 (41.7)	3.328 (0.172)	*AG*	38	8512 (1285)	363 (55)	4.29 (0.41)	283 (39)	3.33 (0.15)
	AA	1	8279 (0.0)	345.0 (0.0)	4.170 (0.0)	265.0 (0.0)	3.210 (0.0)	*AA*	434	8458 (1409)	361 (59)	4.29 (0.46)	281 (43)	3.33 (0.20)
II	*GG*	262	10,730 (1954.2)	448.1 (82.9)	4.204 (0.556)	350.5 (61.4)	3.271 (0.192)	-	-	-	-	-	-	-
	AG	22	11,218.5 (2039.9)	492.0 (78.0)	4.449 (0.619)	365.5 (63.9)	3.264 (0.118)	*AG*	20	11590 (2296)	495 (97)	4.31 (0.59)	374 (66)	3.24 (0.18)
	AA	1	10,485 (0.0)	502.0 (0.0)	4.780 (0.0)	324.0 (0.0)	3.090 (0.0)	*AA*	265	10705 (1921)	448 (81)	4.22 (0.56)	350 (61)	3.27 (0.19)
III	*GG*	132	11,214 (2306.6)	465.7 (100.7)	4.176 (0.572)	363.4 (73.9)	3.247 (0.213)	-	-	-	-	-	-	-
	AG	10	11,804 (2967.8)	495.7 (104.5)	4.263 (0.517)	388.2 (83.5)	3.327 (0.269)	*AG*	10	11709 (3191)	477 (104)	4.14 (0.39)	383 (89)	3.32 (0.26)
	AA	1	10,059 (0.0)	446.0 (0.0)	4.430 (0.0)	317.0 (0.0)	3.150 (0.0)	*AA*	133	11213 (2281)	467 (101)	4.19 (0.58)	363 (74)	3.25 (0.21)

L - lactation

In the case of *ER1/Bgl*I and *ER1/Sna*BI genotypes, the milk yield was highest in the 2nd and 3rd lactation for *AG* genotypes and in the 1st lactation for *GG* genotype. The smallest differences were noticed between *GG*, *AG*, and *AA ER1/Bgl*I genotypes and no statistically significant differences were obtained. For fat yield, the *AA ER1/Bgl*I genotype in the 2nd lactation was the most favorable one. In the 1st and 3rd lactations, fat yield values were higher for *GG* genotype and *AG* genotype, respectively. For protein yield, higher values were observed for *GG* genotype in the 1st lactation and *AG* genotypes in the 2nd and 3rd lactations. None of the analyzed differences were statistically significant.

For the milk, fat, and protein yield in all the lactations, *AG ER1/Sna*BI genotypes were the most favorable ones, although the differences between *AG* and *AA ER1/Sna*BI genotypes were small and statistically non-significant.

Discussion

Marker-assisted selection (MAS) in conjunction with traditional selection methods has been implemented to increase milk production traits (16). All 3 genes examined in this study are strictly related to hormonal metabolism, physiology of reproduction and all of those genes are predicted to be markers for milk production traits. That is why we chose those genes for this kind of experiment.

Expression of *CYP19* gene in placenta is dependent on promoter 1.1. We suggested that variation in the promoter region (P1.1) of *CYP19* may affect milk yield. Frequencies of *CYP19/Pvu*II alleles obtained in the present study for B&W cattle were similar to those obtained by Komisarek and Dorynek (9) and Kowalewska-Luczak (18) for HF cattle. This result confirms our previous study, where the obtained frequencies were 0.947 for allele *A* and 0.053 for allele *B* (19). Higher frequencies of the $CYP19^{B}$ (0.12) were noticed by Vanselow et al. (3). For *CYP19/Pvu*II polymorphism, the *AA* genotypes were favorable.

For bovine ER1 gene, several nucleotide sequence polymorphisms were found. The polymorphic sites were located within the 5' promoter region of this gene. One polymorphic site is recognized by *Bgl*I enzyme and it is an A/G transition. The second one is an A/G transition in promoter B, recognized by SnaBI and the third one is an A/C transversion at position +503 (within exon I) recognized by *Tsp*RI (20). Our analysis focused on 2 polymorphic sites within promoter regions. The genotype frequencies for *Bgl*I and *Sna*BI observed in our study were similar to those obtained by Szreder and Zwierzchowski (15), and Szreder et al. (17,21). For *Sna*BI polymorphism, *AG* genotype was the most favorable one with higher milk, fat, and protein yields. The analysis of *ER1/Bgl*I polymorphism shows higher levels of milk production traits for *AG* and *GG* genotypes.

It is known that all eukaryote genomes contain repetitive dispersed sequences, e.g. endogenous retrovirus, LINEs, and SINEs. In *Bovidae*, short interspersed nucleotide elements, especially the Bov-A, are rather frequent. This kind of sequence was probably generated by the deletion of a central part of the Bov-B LINE (14,22). The retroelements influence the host genome and might have played an important role in the concentrations and production of new polymorphisms (14). In the promoter region of the bovine *CYP21* gene, the Bov-A2 SINE was localized and was analyzed using *Hpa*II restriction endonuclease (13). After the analysis of all the facts presented by Damiani et al. (13,14), we predicted to find polymorphic site within promoter region of *CYP21* gene recognized by *Hpa*II restriction endonuclease. In the analyzed fragment of the promoter region of the *CYP21* gene, one *AA* genotype was observed. It is possible that for other breeds this polymorphic site would be observed. The examination should also be evaluated further using other population or higher animal numbers.

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